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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
TALAVERA, MIGUEL A	

ART UNIT	PAPER NUMBER
1656	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/601,324	CRONIN ET AL.	
	Examiner	Art Unit	
	Miguel A. Talavera	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *31 January 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-25* is/are pending in the application.

4a) Of the above claim(s) *18-25* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-16* is/are rejected.

7)☒ Claim(s) *17* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *20 June 2003* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *Appendix A*.

Application Status

1. Claims 1-25 are pending in the application.

2. Applicant's amendment to the claims, filed on January 31, 2006, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

3. Applicant's election with traverse of Group I, claims 1-17, in the reply filed on January 31, 2006 is acknowledged. The traversal is on the ground(s) that "that the basis for restriction pursuant M.P.E.P. § 803 has not been met". Specifically, applicant argues that because each of inventions I and II includes the limitation of a protein that has at least 90% identity to amino acids 605-883 of SEQ ID NO:1, "there is a clear relationship between inventions I and II." This is not found persuasive. Initially is noted that the claims in Group I are drawn to compositions comprising a protein in crystalline form and methods of making said protein crystals, whereas claims in Group II are drawn to methods of identifying an associating species requiring the use of the structural coordinates of a protein. As stated in the Office Action of December 15, 2005, the crystals of Group I have additional utilities and are not required to practice the method of Group II. Those of skill in the art understand that a macromolecular crystal can diffract an X-ray beam into a pattern of reflections that can (through crystallographic data analysis) produce a three dimensional map of the macromolecule's electron density from which structure coordinates can be calculated. Therefore, the structure coordinates that can be obtained from the crystals of Group I are physically different from the composition comprising a protein in crystalline form of Group I, as described above. Although the claimed products of Group I are related to the claimed methods of Group II by virtue of the structural coordinates that can potentially be obtained, they are distinct because the inventions as claimed do not overlap in scope, i.e., are mutually exclusive; the inventions as

claimed are not obvious variants; and the inventions as claimed have a materially different design, mode of operation, function, and effect. See MPEP § 806.05(j). That each Group is distinct is evidenced by their separate status in the art and their separate classification. Thus, there is a search burden on the examiner to examine all groups together. Thus, the restriction is deemed proper and is therefore made FINAL.

4. Claims 18-25 are withdrawn from further consideration pursuant to 37 C.F.R. § 1.142(b), as being drawn to a nonelected invention(s), there being no allowable generic or linking claim. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

5. Claims 1-17 are being examined on the merits.

Priority

6. Applicants' claim to domestic priority under 35 U.S.C. § 119(e) to US provisional application 60/390356, filed on June 21, 2002, is acknowledged. The claimed invention finds support in the provisional application.

Information Disclosure Statement

7. The Examiner can find no information disclosure statement (IDS) filed in the instant application. If the Examiner has inadvertently overlooked an IDS that has been previously filed in the instant application, applicants' cooperation is requested in alerting the examiner to this IDS in the response to this Office action.

Compliance with the Sequence Rules

8. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequence set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. § 1.821 through 1.825; applicants' attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990) and 1114 OG 29 (May 15, 1990).

a) The structural coordinates in Figures 3A and 3B teach an amino acid sequence since a particular amino acid is assigned to a linear sequence in a particular order. As such, the amino acid sequence disclosed within the atomic coordinates must comply with the sequence rules. Labeling using a SEQ ID NO must be inserted into the brief description of the drawings or into the Figure directly.

If the noted sequences are in the sequence listing as filed, Applicants must amend the specification to identify the sequences appropriately by SEQ ID NO. If the noted sequences are not in the sequence listing as filed, Applicants must provide (1) a substitute copy of the sequence listing in both computer readable form (CRF) and paper copy, (2) an amendment directing its

entry into the specification, (3) a statement that the content of the paper and CRF copies are the same and, where applicable, include no new matter as required by 37 C.F.R. § 1.821 (e) or 1.821(f) or 1.821(g) or 1.821(b) or 1.825(d), and (4) any amendment to the specification to identify the sequences appropriately by SEQ ID NO.

Objections to the Drawings

9. The drawings are objected to because the Figures are not numbered in accordance with 37 CFR 1.84(u)(1), which states, "[p]artial views intended to form one complete view, on one or several sheets, must be identified by the same number followed by a capital letter." A proposed drawing correction or corrected drawings are required in reply to the Office action to avoid abandonment of the application. The objection to the drawings will not be held in abeyance.

Objections to the Specification

10. The specification is objected to because the title is not descriptive. A new title is required that is clearly indicative of the invention to which the elected claims are drawn (see M.P.E.P. § 606.01). The Examiner suggests the following new title:

---Crystalline form of the wild-type kinase domain of human Ephrin Receptor A2 (EPHA2) in complex with AMP-PNP---

11. In the specification, the Abstract is objected to for not completely describing the disclosed subject matter (see M.P.E.P. § 608.01(b)). It is noted that in many databases and in foreign countries, the Abstract is crucial in defining the disclosed subject matter, thus, its

completeness is essential. The Examiner suggests inclusion of the name of the source species (i.e. human), for completeness.

Claim Objections

12. Claims 1-3, 9-11, and 16-17 are objected to as reciting the improper sequence identifier "SEQ. ID No.," which should be replaced with "SEQ ID NO:". See 37 C.F.R. § 1.821(d).

13. Claims 7-8 are objected to because of the recitation of "EPHA2." Abbreviations, unless otherwise obvious and/or commonly used in the art, *e.g.,* DNA, should not be recited in the claims without at least once reciting the entire phrase for which the abbreviation is used. Appropriate correction is required.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 4, 7, 8 and 12 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

14. The phrase "a resolution greater than 3.0 Angstroms", in claims 4 and 12, are unclear as to the limitations they impart on the claimed subject matter or as to what said phrases encompass and one of skill in the art would not be reasonably apprised of the scope of the claimed crystals.

It is well known in the art that the smaller the number (i.e. Angstroms), the higher the resolution. In this case, the term "greater than" with respect to 3.0 Angstroms can be interpreted in the following two distinct ways: 1) a *number* greater than 3.0 Å, *i.e.,* a lower resolution, or 2) a *resolution* greater than 3.0 Å, *i.e.,* a higher resolution. Clarification is required.

15. Claims 7 and 8 recite the abbreviation "EPHA2" and it is unclear from the specification and the claims as to the scope of polypeptides that are intended as being encompassed by "EPHA2." Neither the specification nor the claims teach those identifying characteristics that distinguish an "EPHA2" polypeptide from other tyrosine kinases. The application teaches many properties of an "EPHA2A" polypeptide (beginning at p. 13), but fails to define which of these properties or features are necessary for inclusion of a tyrosine kinase within the scope of the claims. Clarification of the term "EPHA2" is suggested.

16. Claim 16 is confusing in the recitation of "protein expressed as SEQ ID No. 2" as SEQ ID NO:2 is disclosed as being a nucleic acid and not a polypeptide. It is suggested that applicant replace the term with, for example, "protein encoded by SEQ ID NO:2." In the interest of advancing prosecution, the examiner has interpreted the term as meaning protein encoded by SEQ ID NO:2.

Claim Rejections - 35 U.S.C. § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention

Written Description

17. Claims 1-16 are rejected under 35 U.S.C. § 112, first paragraph, written description, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The instant claims are drawn to genera of compositions containing crystalline Ephrin receptor A2 (EPHA2) and methods of making said compositions and a genus of compositions comprising at least a portion of SEQ ID NO:2. Specifically, claims 1-15 are directed to compositions consisting of all possible crystals and methods of making said crystals that contain a genera of polypeptides comprising polypeptides having "at least 90% identity with the amino acid with residues 605-883 of SEQ. ID No. 1" (Claims 1 and 9) with optional additional limitations presented in individual, dependent claim form such as: at least 95% identity with residues 605-883 of SEQ. ID No. 1 (Claims 2 and 10), comprises consecutively of residues 605-883 of SEQ. ID No. 1 (Claims 3 and 11), a certain resolution (Claims 4 and 12), a $P3_221$ space group (Claims 5 and 10), certain unit cell dimensions (claims 6 and 14) and producing a diffraction pattern suitable for protein structure determination (claim 15). To clarify the record, Applicants state at p.21, §0092, [i]t should be understood that forming crystals comprising EPHA2 and crystals comprising EPHA2 according to the invention are not intended to be limited to the wild-type, full-length length EPHA2 shown in SEQ ID NO: 1 and to fragments comprising residues 572-976, 596-900, or 605-883 of SEQ ID NO: 1. Rather, it should be recognized that the invention may be extended to various other fragments and variants of wild-type EPHA2 as described above.] As such, included in the claimed crystalline form genus are any fragments and variants of EPHA2 (claims

7 and 8), wherein said variants can be made by any known genetic engineering method or obtained from any source known in the art. Finally, the claimed compositions also include a genus of polypeptides comprising at least a portion of a protein encoded by SEQ ID NO: 2 (claim 16). The latter compositions are widely variant ranging from those containing a polypeptide comprising a single amino acid of the polypeptide encoded by SEQ ID NO:2 to those containing the full-length protein encoded by SEQ ID NO: 2 fused to anything known in the art.

While the specification describes isolation of *human* EPHA2 having the amino acid sequence of SEQ ID NO: 3 (residues 1-28 are presumably the polyhistidine tag that was not removed, see p. 48, ¶[00200], and residues 29-333 of SEQ ID NO:3 are equivalent to residues 596-900 of SEQ ID No: 1), a crystal form of SEQ ID NO:3 in complex with AMP-PNP with space group $P3_221$ and unit cell dimensions a= 72.12 Å, b= 72.12 Å, c= 241.62 Å (see specification, page 25, Table 5) and X-ray crystallographic analysis leading to the structural coordinates of said polypeptide in complex with AMP-PNP as shown in Figures 3A and 3B, this single disclosed crystal species fail to represent the variation among the species of claimed crystalline compositions, which encompasses a crystal of any other polypeptide having at least 90% identity with the amino acid with residues 605-883 of SEQ. ID No. 1, including crystals of polypeptides having any function. Similarly, the specification discloses structure and function of two species, SEQ ID NOs: 1 and 3, comprising at least a portion of the protein encoded by SEQ ID No: 2, but the structural and functional features of all compositions containing polypeptides comprising at least a portion of the protein encoded by SEQ ID No: 2 are not described.

The Court of Appeals for the Federal Circuit has recently held that a "written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as be structure, formula [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." University of California v. Eli Lilly and Co., 1997 U.S. App. LEXIS 18221, at *23, quoting Fiers v. Revel, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) (bracketed material in original). To fully describe a genus of genetic material, which is a chemical compound, applicants must (1) fully describe at least one species of the claimed genus sufficient to represent said genus whereby a skilled artisan, in view of the prior art, could predict the structure of other species encompassed by the claimed genus and (2) identify the common characteristics of the claimed molecules, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these (Enzo Biochem 63 USPQ2d 1609 (CAFC 2002)).

Regarding the methods of claims 9-15, *University of Rochester v. G.D. Searle & Co.* (69 USPQ2d 1886 (2004)) specifically points to the applicability of both *Lily* and *Enzo Biochemical* to methods of using products, wherein said products lack adequate written description. While in *University of Rochester v. G.D. Searle & Co.* the methods were held to lack written description because not a single example of the product used in the claimed methods was described, the same analysis applies wherein the product, used in the claimed methods, must have adequate written description as noted from *Enzo Biochemical* (see above).

At the time of the invention, it was well known in the art that polypeptides, such as any EPHA2 protein or any polypeptide having at least 90% identity to a reference amino acid sequence, such as SEQ ID NO: 1, were diverse in sequence. Such heterogeneous chemical composition of proteins leads to a diverse population of amino acid sequences whose particular crystallization conditions are unique. Similarly, it was well known in the art that a macromolecular crystal was defined by three repeating vectors a, b, and c, with **angles α, β, and γ, between them**. See pp. 586 and 2725 of the "Encyclopedia of Molecular Biology" (Creighton, T., John Wiley and Sons, Inc. New York, 1999).

In general, for a species of crystal to be adequately structurally described, the following must be adequately disclosed: (1) the composition of the crystal (exact structural features of all molecules in the crystal must be described, including the protein (preferably a SEQ ID NO of all included residues) and any molecule bound to it), (2) the space group, and (3) the unit cell dimensions of the crystal. The species noted above has partially met this burden by the description in the instant specification. Still needed is a description of the angles angles α, β, and γ. However, the composition of the crystals encompassed by the breadth of the claims is not described because the exact molecule is not limited nor the space group and unit cell dimensions associated with this breadth of chemical composition described. For example, in claims 6 and 14, only unit cell dimensions are adequately described. The exact polypeptide sequence (SEQ ID No. 1) accompanied by the recitations "comprises", "at least a portion" or "EPHA2"(see the interpretation set forth above) and percent identity language does not disclose the exact composition of the protein crystal in claims 1-3 and 7-11. The space group disclosed in Claims 5 and 13 satisfies one criterion for adequate description but missing the other two criteria for

description as noted above. A singular chemical composition has unique crystallization parameters that lead to a crystal whose space group and unit cell dimensions can only be determined by analyzing that crystal's X-ray diffraction and not inferred *a piori* (Giege *et al.* Crystallogenesis of Biological Macromolecules: Facts and Perspectives. Acta Cryst., (1994) D50: 339-350). Since our understanding of crystallization mechanisms are still incomplete and the factors of macromolecular structure that are involved in crystallization are poorly understood, a method of the crystallization encompassed by the breadth of the claims is not adequately described by the method of crystallization disclosed in the specification. Therefore, the conditions disclosed in the specification to crystallize SEQ ID No. 3 cannot sufficiently describe "conditions suitable" for instant genus.

Given the lack of description of a representative number of macromolecular crystals or compositions containing any other polypeptide as encompassed by the claims the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicant was in possession of the claimed invention.

Scope of Enablement

12. Claims 1-16 are rejected under 35 U.S.C. § 112, first paragraph, scope of enablement, because the specification, while being enabling for a crystal of SEQ ID NO: 3 in complex with AMP-PNP with space group $P3_221$ and unit cell dimensions a= 72.12 Å, b= 72.12 Å, c= 241.62 Å (see specification, page 25, Table 5) and prepared under the crystallizations conditions at p. 49, ¶¶[00203]-[00204] and a composition comprising SEQ ID NO:1 or 3, does not reasonably provide enablement for crystals containing any EPAH2 or any polypeptide having "at least 90%

identity with the amino acid with residues 605-883 of SEQ. ID No. 1" and methods of preparation thereof as broadly encompassed by the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. To practice the claimed methods to the full extent of their scope would require undue experimentation.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See M.P.E.P. § 2164.01(a). The Factors most relevant to the instant rejection are addressed in detail below.

The breadth of the claims: Claims 1 (claims 2-6 dependent therefrom), 7 and 8 are so broad to encompass compositions comprising all possible crystals and methods of making said crystals that contain polypeptides comprising any EPHA2 or polypeptides having "at least 90% identity with the amino acid with residues 605-883 of SEQ. ID No. 1" (Claims 1 and 9) with optional additional limitations presented in individual, dependent claim form such as: at least 95% identity with residues 605-883 of SEQ. ID No. 1 (Claims 2 and 10), comprises consecutively of residues 605-883 of SEQ. ID No. 1 (Claims 3 and 11), a certain resolution (Claims 4 and 12), a $P3_221$ space group (Claims 5 and 10), with protein crystals with unit cell dimensions of +/- 5%, of a=72.12 Å, b=72.12 Å, c=241.62 Å (claims 6 and 14) and producing a

diffraction pattern suitable for protein structure determination (claim 15). According to the interpretation provided above regarding "EPAH2" the claimed crystals include those comprising all variants that have been manipulated or reengineered for crystallization purposes. Claim 16 is so broad as to encompass any polypeptide comprising at least a portion of a protein encoded by SEQ ID NO: 2. A "portion of a protein encoded by SEQ ID NO:2" has been interpreted as meaning any fragment of a protein encoded by SEQ ID NO:2, including a single amino acid. Thus, in accordance with MPEP 2111, claim 16 has been interpreted as being drawn to a composition comprising any polypeptide having any function. The broad scope of claimed crystals is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of compositions comprising the protein crystals and polypeptides as broadly encompassed by the instant claims. In this case the disclosure is limited to enabling a crystal of SEQ ID NO: 3 in complex with AMP-PNP and the polypeptide encoded by SEQ ID NO: 2. The specification teaches (specification at p. 49, ¶¶[00204] and [00205]) that crystallization of SEQ ID NO: 3 in complex with AMP-PNP was obtained as follows:

> "Diffraction quality crystals were grown as in 100nL sitting droplets using the vapor diffusion method. 50nL comprising the EPHA2-AMP-PNP complex (6.1mg/ml) was mixed with 50nL from a reservoir solution (100μL) comprising 0.1M Citrate/Acetate pH=5.0 and 24% PEG MME 2K. The resulting solution was incubated over a period of one week at 20 C.
>
> Crystals typically appeared after 8-24 hours and grew to a maximum size within 48 hours. Single crystals were separated from their parent cluster and transferred, briefly, into a cryoprotecting solution containing the reservoir solution supplemented with 20%, v/v ethylene glycol. Crystals were then flash frozen by immersion in liquid nitrogen and then stored under liquid nitrogen. A crystal of EPHAZ-AMP-PNP complex produced as described is illustrated in Figure 2."

The nature of the invention: The invention of claims 1-15 is related to protein crystals of the kinase domain of human EPHA2 in complex with AMP-PNP. At the time of the invention,

methods of macromolecular crystallization were well known in the art. However, the ability to crystallize a given macromolecule was, at the least, challenging to a skilled artisan as even minor alterations in the conditions of crystallization could result in altered crystal forms, crystals of sub-diffraction quality, or a lack of crystal growth. The invention of claim 16 is a composition comprising a polypeptide.

The state of the prior art; The level of one of ordinary skill; and The level of predictability in the art: Regarding the claimed crystals and methods for making same, the state of the art at the time of the invention acknowledges a high level of unpredictability for making the full scope of claimed crystals. It is well known in the art of protein chemistry that crystallizing a macromolecule is a chancy and difficult process without any clear expectation of success. It is now evident that protein crystallization is the main hurdle in protein structure determination. For this reason, protein crystallization has become a research subject in and of itself, and is not simply an extension of the structural biologist or crystallographer's laboratory. There are many references that describe the difficulties associated with growing protein crystals. See for example, Kierzek *et al.*, Biophys. Chem., vol. 91, pages 1-20, 2001, Wiencek, Annu. Rev. Biomed. Eng., vol. 1, pages 505-534, 1999 and Ke & Doudna, Methods, vol. 34, pp. 408-414, 2004.

> "Crystallization is predictably the least predictable aspect of a structure determination project" (emphais added; see Ke & Doudna, page 408)

It is commonly held that crystallization of macromolecules from solution is the major obstacle in the process of determining macromolecular structures. The reasons for this are many; biological macromolecules are complex, and the delicate balance involving specific and non-

specific interactions with other macromolecules and small molecules in solution, is difficult to predict.

Each biological macromolecule crystallizes under a unique set of conditions, which cannot be predicted in advance. Simply supersaturating the macromolecule to bring it out of solution may not work, the result would, in most cases, be an amorphous precipitate. Many precipitating agents are used, common ones are different salts, and polyethylene glycols, but others are known. In addition, additives such as metals and detergents can be added to modulate the behavior of the macromolecule in solution. Many kits are available (e.g. from Hampton Research ©), which attempt to cover as many parameters in crystallization space as possible, but in many cases these are just a starting point to optimize crystalline precipitates and crystals which are unsuitable for diffraction analysis. Likewise, known crystallization conditions of another macromolecule with sequence identity or similar fold as the target macromolecule for crystallization are also often regarded as a starting point of parameters in crystallization space. Successful crystallization is aided by knowledge of the macromolecules behavior in terms of solubility, dependence on metal ions for correct folding or activity, interactions with other molecules and any other information that is available. As evidenced by Derewenda *et al.* (Acta Crystallogr. D., vol. 62, pages 116-124, 2006) the outcome of macromolecular crystallization is further compounded by the chemical composition of the macromolecule itself, in particular the molecular surface area, and available surface sites that might participate (i.e., crystal contacts) in holding together the three-dimensional array of macromolecules defining the crystal lattice:

> "Clearly, the protein's microscopic surface properties have a critical impact on the thermodynamics and kinetics of crystallization. It follows then that some proteins will crystallize more easily than others and that the amino-acid composition and

> sequence are more informative with respect to possible crystallization outcome than is normally believed."(emphasis added; see Derewenda *et al.*)

Because functional variants within the claimed polypeptide genus, i.e, polypeptides having at least 90% identity with residues 605-883 of SEQ ID NO: 1, might differ primarily in their molecular surfaces and not at the catalytic cores required for the enzymatic function, said functional variants are unpredictable to crystallize because one can not predict the particular juxtaposition of functional groups of neighboring molecules that would lead to crystal contacts.

> "It is arguable that homolog screening is a relic of the bygone era when it was the only means of diversifying the protein sample. In reality, it suffers from a major limitation: the crystallizability of any given homolog is as unpredictable as that of the original target." (emphasis added ; see Derewenda *et al.*)

Further, it was also well known in the art that the specific chemical composition largely defines crystallization success of any crystal of a variant, such as those comprising "EPHA2" or any polypeptide having at least 90% identity with residues 605-883 of SEQ ID NO: 1. The general knowledge in the art, as exemplified by Buts *et al.* (Acta Crystallogr. D., vol. 61, pages 1149-1159, 2005), regarding the impact of the protein sequence of a macromolecule on crystallization behavior, states:

> "Since the introduction of structural genomics, the protein has been recognized as the most important variable in crystallization." "Five naturally occurring variants, differing in 1-18 amino acids, of the 177-residue lectin domain of the F17G fimbrial adhesin were expressed and purified in identical ways. For four out of the five variants crystals were obtained, mostly in non-isomorphous space groups, with diffraction limits ranging between 2.4 and 1.1 Å resolution."

Specifically, the reference of Buts *et al.* teaches that the F17e-G and F17f-G adhesins differ in only one amino acid from the F17c-G adhesin, Arg21Ser and His36Tyr, respectively, and yet

these proteins that are 99% identical in sequence resulted in different crystal forms with distinct diffraction properties (see Tables 1-3).

Since our understanding of crystallization mechanisms are still incomplete and the factors of macromolecular structure that are involved in crystallization are poorly understood, to make the macromolecular crystals encompassed by the scope of the genus of instant claims, the following must be clear: the preparation and chemical composition of the molecules to be crystallized –including ligands--, and the crystallization conditions, including methods and reagents used. Crystallization experiments must be done in order to empirically determine if a macromolecule will crystallize, and preliminary X-ray diffraction experiments must be done in order to determine if the crystalline macromolecule will diffract to the resolution required for analysis. Therefore, precise instruction about how to make macromolecular crystals suitable for structure determination is required so that undue experimentation is not required.

It is the Examiner's position that screening for suitable variants with at least 90% percent identity to SEQ ID NO: 1 leading to diffraction quality crystals would constitute undue experimentation. Furthermore, it is highly unpredictable obtaining crystals of a variant having an unspecified sequence composition with an expectation that said crystals have the same intrinsic parameters such as space group (Claims 7 and 13), unit cell dimensions (Claims 6, 8 and 14), diffraction limit (Claims 4 and 12), or diffraction data quality that would enable structure determination (see claim 15) as those for a known crystal form of a specific polypeptide (i.e. SEQ ID NO: 3).

In view of these teachings, a skilled artisan would recognize that it is highly unpredictable as to whether diffraction-quality crystals of other EPHA2 polypeptides as

encompass by the claims can be achieved using the crystallization parameters of SEQ ID NO: 3 in complex with AMP-PNP as set forth on p. 49 of the specification. Since determination of unit cell dimensions, space group, diffraction limit and data quality necessitate the diffraction data from a crystal, a skilled artisan would recognize that it is highly unpredictable to know *a priori* if any other variant of EPHA2 would form crystals having the specified claimed limitations. That is, current macromolecular structure prediction is not accurate enough nor can macromolecule-solvent and macromolecule-macromolecule interactions be modeled with the necessary precision to pinpoint all contributions to the free energy of crystallization, *ab initio* crystallization prediction for macromolecules is not feasible (see Kierzek *et al.*).

Regarding the polypeptide of claim 16, the state of the art at the time of the invention acknowledges a high level of unpredictability in making a variant of SEQ ID NO:3 with an expectation that the polypeptide would maintain the desired activity/utility. For example, the prior art reference of Witkowski et al. (*Biochemistry* 38:11643-11650) teaches that even a single amino acid substitution can substantially alter a protein's activity, in the case of Witkowski et al., changing a beta-ketoacyl synthase to a malonyl decarboxylase (p. 11647, Table 1).

The amount of direction provided by the inventor; The existence of working examples: The specification discloses the utility of the claimed crystal is in the determination of the 3-D structure of EPHA2 (p. 2, ¶[006]). The specification discloses only one working example of a diffraction-quality crystal. As there is no evidence in either the specification or the prior art that the disclosed crystallization conditions are AMP-PNP dependent, it should be noted that the working example might be specific to a binary complex between SEQ ID NO: 3 and AMP-PNP. Other than this working example, the specification fails to provide guidance for altering the

crystallization conditions for crystallizing any other polypeptides comprising a variant of EPHA2 or having at least 90% percent identity with residues 605-883 of SEQ ID NO: 1 with an expectation of obtaining diffraction-quality crystals or any other conditions with an expectation of obtaining diffraction-quality crystals. Similarly, the specification discloses only two working examples of polypeptides as encompassed by claim 16, *i.e.,* SEQ ID NO:1 and 3. Other than these examples, the specification fails to provide any guidance for altering the polypeptide of SEQ ID NO:1 or 3 with an expectation that the polypeptide will maintain the desired activity/utility.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure: While methods of macromolecular crystallization were known at the time of the invention, these methods are specific to a particular macromolecule as evidenced by the above teachings. Thus, a skilled artisan is left to experiment by a trial and error process to determine whether the disclosed crystallization conditions can be applied to crystallization of polypeptides encompassed by the claims or whether said polypeptides can be crystallized at all. Also, while methods of generating variants of a polypeptide were known in the art at the time of the invention, it was not routine in the art to screen all polypeptides having a substantial number of modifications and having any activity as encompassed by the claims for those having the desired activity/utility.

In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, the high level of unpredictability as evidenced by the prior and current state of the art, and the amount of experimentation required to make all the crystals and polypeptides as broadly encompassed by the claims, undue experimentation would

be necessary for a skilled artisan to make and use the entire scope of the claimed invention. Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 U.S.C. § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action

> b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

18. Claims 16 is rejected under 35 U.S.C. § 102(b) as being anticipated by Lindberg *et al.* (Mol. Cell. Biol., vol. 10, pp. 6316-24, 1990). Claim 16 is drawn to a polypeptide comprising at least a portion of a protein encoded by SEQ ID NO: 2. A "portion of a protein encoded by SEQ ID NO:2" has been interpreted as meaning any fragment of a polypeptide encoded by SEQ ID NO:2, including a single amino acid. Thus, in accordance with MPEP 2111, claim 16 has been interpreted as being drawn to a composition comprising any polypeptide having any function.

Lindberg *et al.* teach a polypeptide that is 100% identical to a polypeptide encoded by

SEQ ID NO:2 herein (see Appendix A and p. 6318, Figure 1 of Lindgerg et al.). This anticipates claim 16 as written.

Conclusion

Claims 1-17 are rejected for the reasons identified in the numbered sections of the Office action. Applicants must respond to the objections/rejections in each of the numbered sections in the Office action to be fully responsive in prosecution.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Miguel A. Talavera whose telephone number is (571)272-3354. The examiner can normally be reached on M-F, 8:30am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen M. Kerr can be reached on (571)272-0931. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Miguel A. Talavera, Ph.D.
March 23, 2006

DAVID J. STEADMAN, PH.D.
PRIMARY EXAMINER

Appendix A-Sequence Alignment

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RESULT 1
A36355
protein-tyrosine kinase (EC 2.7.1.112) eck precursor - human
C;Species: Homo sapiens (man)
C;Date: 28-Mar-1991 #sequence_revision 28-Mar-1991 #text_change 09-Jul-2004
C;Accession: A36355
R;Lindberg, R.A.; Hunter, T.
Mol. Cell. Biol. 10, 6316-6324, 1990
A;Title: cDNA cloning and characterization of eck, an epithelial cell receptor
protein-tyrosine kinase in the eph/elk family of protein kinases.
A;Reference number: A36355; MUID:91061738; PMID:2174105
A;Accession: A36355
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-976
A;Cross-references: UNIPROT:P29317; UNIPARC:UPI000012A070; GB:M59371; GB:M36395;
NID:g181943; PIDN:AAA53375.1; PID:g181944
C;Genetics:
A;Gene: GDB:ECK
A;Cross-references: GDB:126726
A;Map position: 1p36-1p36
C;Superfamily: protein-tyrosine kinase, receptor type eph; fibronectin type III repeat
homology; protein kinase homology; SAM homology
C;Keywords: ATP; autophosphorylation; phosphoprotein; phosphotransferase;
transmembrane protein; tyrosine-specific protein kinase
F;611-878/Domain: protein kinase homology
F;619-627/Region: protein kinase ATP-binding motif
F;901-967/Domain: SAM homology

Alignment Scores:
Pred. No.:              1.42e-78        Length:         976
Score:                  1590.00         Matches:        305
Percent Similarity:     100.0%          Conservative:   0
Best Local Similarity:  100.0%          Mismatches:     0
Query Match:            91.3%           Indels:         0
DB:                     2               Gaps:           0

US-10-601-324-2 (1-915) x A36355 (1-976)

Qy          1 GACCCCAACCAGGCTGTGTTGAAGTTCACTACCGAGATCCATCCATCCTGTGTCACTCGG 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        596 AspProAsnGlnAlaValLeuLysPheThrThrGluIleHisProSerCysValThrArg 615

Qy         61 CAGAAGGTGATCGGAGCAGGAGAGTTTGGGGAGGTGTACAAGGGCATGCTGAAGACATCC 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        616 GlnLysValIleGlyAlaGlyGluPheGlyGluValTyrLysGlyMetLeuLysThrSer 635

Qy        121 TCGGGGAAGAAGGAGGTGCCGGTGGCCATCAAGACGCTGAAAGCCGGCTACACAGAGAAG 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        636 SerGlyLysLysGluValProValAlaIleLysThrLeuLysAlaGlyTyrThrGluLys 655

Qy        181 CAGCGAGTGGACTTCCTCGGCGAGGCCGGCATCATGGGCCAGTTCAGCCACCACAACATC 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        656 GlnArgValAspPheLeuGlyGluAlaGlyIleMetGlyGlnPheSerHisHisAsnIle 675

Qy        241 ATCCGCCTAGAGGGCGTCATCTCCAAATACAAGCCCATGATGATCATCACTGAGTACATG 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Db        676 IleArgLeuGluGlyValIleSerLysTyrLysProMetMetIleIleThrGluTyrMet 695

Qy        301 GAGAATGGGGCCCTGGACAAGTTCCTTCGGGAGAAGGATGGCGAGTTCAGCGTGCTGCAG 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        696 GluAsnGlyAlaLeuAspLysPheLeuArgGluLysAspGlyGluPheSerValLeuGln 715

Qy        361 CTGGTGGGCATGCTGCGGGGCATCGCAGCTGGCATGAAGTACCTGGCCAACATGAACTAT 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        716 LeuValGlyMetLeuArgGlyIleAlaAlaGlyMetLysTyrLeuAlaAsnMetAsnTyr 735

Qy        421 GTGCACCGTGACCTGGCTGCCCGCAACATCCTCGTCAACAGCAACCTGGTCTGCAAGGTG 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        736 ValHisArgAspLeuAlaAlaArgAsnIleLeuValAsnSerAsnLeuValCysLysVal 755

Qy        481 TCTGACTTTGGCCTGTCCCGCGTGCTGGAGGACGACCCCGAGGCCACCTACACCACCAGT 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        756 SerAspPheGlyLeuSerArgValLeuGluAspAspProGluAlaThrTyrThrThrSer 775

Qy        541 GGCGGCAAGATCCCCATCCGCTGGACCGCCCCGGAGGCCATTTCCTACCGGAAGTTCACC 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        776 GlyGlyLysIleProIleArgTrpThrAlaProGluAlaIleSerTyrArgLysPheThr 795

Qy        601 TCTGCCAGCGACGTGTGGAGCTTTGGCATTGTCATGTGGGAGGTGATGACCTATGGCGAG 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        796 SerAlaSerAspValTrpSerPheGlyIleValMetTrpGluValMetThrTyrGlyGlu 815

Qy        661 CGGCCCTACTGGGAGTTGTCCAACCACGAGGTGATGAAAGCCATCAATGATGGCTTCCGG 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        816 ArgProTyrTrpGluLeuSerAsnHisGluValMetLysAlaIleAsnAspGlyPheArg 835

Qy        721 CTCCCCACACCCATGGACTGCCCCTCCGCCATCTACCAGCTCATGATGCAGTGCTGGCAG 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        836 LeuProThrProMetAspCysProSerAlaIleTyrGlnLeuMetMetGlnCysTrpGln 855

Qy        781 CAGGAGCGTGCCCGCCGCCCCAAGTTCGCTGACATCGTCAGCATCCTGGACAAGCTCATT 840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        856 GlnGluArgAlaArgArgProLysPheAlaAspIleValSerIleLeuAspLysLeuIle 875

Qy        841 CGTGCCCCTGACTCCCTCAAGACCCTGGCTGACTTTGACCCCCGCGTGTCTATCCGGCTC 900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        876 ArgAlaProAspSerLeuLysThrLeuAlaAspPheAspProArgValSerIleArgLeu 895

Qy        901 CCCAGCACGAGCGGC 915
              |||||||||||||||
Db        896 ProSerThrSerGly 900
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